Kinin generation in acute pneumonia and chronic bronchitis

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ABSTRACT: Kinins are potent inflammatory mediators, liberated from kininogens by different kininogenases. The aim of this study was to investigate the kinin generation pathways in acute and chronic inflammation of the lower airways.

We studied bronchoalveolar lavage fluid (BALF) of patients with acute pneumonia, patients with chronic bronchitis and healthy controls. Kinins were determined by radioimmunoassay (RIA). Plasma kallikrein (pl-Kal), α_2 -macroglobulin (α_2 -M) and toluenesulphonylarginine methyl ester (TAME) esterase activity (TAME-ea) were studied in BALF before and after gel filtration chromatography. Plasma kallikrein and α_2 -M were measured using two newly developed sandwich enzyme-linked immunosorbent assays (ELISAs). TAME-ea was measured by a radiochemical assay. After gel filtration, inhibition of TAME-ea with benzamidine, soy-bean-trypsin inhibitor (SBTI) and aprotinin was performed.

Kinins and TAME-ea did not differ significantly between acute pneumonia and chronic bronchitis, whereas pl-Kal and α_2 -M values were significantly higher in acute pneumonia. Gel filtration revealed the highest TAME-ea peak in acute pneumonia corresponding with the first α_2 -M peak at ~800 kDa, whereas in chronic bronchitis the highest peak was found at ~40 kDa. The inhibition test showed that the TAME-ea peak at ~800 kDa was due to pl-Kal and the TAME-ea peak at ~40 kDa was mainly due to tissue kallikrein. High peaks of α_2 -M and pl-Kal were found in pneumonia and only small peaks were seen in chronic bronchitis.

We conclude that in acute airway inflammation kinins seem to be mainly generated by plasma kallikrein whereas in chronic inflammation, kininogenases other than plasma kallikrein, such as tissue kallikrein, seem to be more important.

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Airway inflammation may be acute, as in pneumonia, when high fever, pain and radiological signs of infiltration are usually present due to bacteria, viruses or mycoplasma. In contrast, chronic bronchitis is characterized by cough and expectoration of mucus over a period of more than 3 months in two consecutive years. Fever is rarely found and is related to bacterial infection. In the process of inflammation many potent inflammatory mediators, such as kinins, are released.

Bradykinin and lysyl-bradykinin are liberated from kininogens by kininogenases, such as plasma kallikrein (pl-Kal) (molecular weight (MW) ~100 kDa) and tissue kallikrein (t-Kal) (MW 2,543 kDa) [1, 2]. Plasma kallikrein liberates bradykinin mainly from high-molecular-weight kininogen (HMW-kininogen), which is present in plasma, but also, to a lesser extent, from low-molecular-weight kininogen (LMW-kininogen). Lysyl-bradykinin is generated by t-Kal, which is secreted by local glands and liberates kinins from both HMW- and LMW-kininogen. Finally, kinins may be generated by tissue proteases other than kallikrein [3–7].

In allergic rhinitis an increase of pl-Kal and t-Kal was found and up to 75% of the kinin-generating activity was shown to be related to pl-Kal [8–11]. Plasma kallikrein is inhibited by several protease inhibitors

including α_2 -macroglobulin (α_2 -M), C1-esterase-inhibitor, antithrombin III and α_1 -antitrypsin [12–14]. α_2 -M, a tetramer with a MW ~725 kDa [15, 16], is a potent inhibitor of many proteases. It forms a complex with the pl-Kal molecule and thus masks its enzymatically active site. In contrast to other inhibitors, the kiningenerating activity of pl-Kal is almost totally inhibited in the α_2 -M/pl-Kal complex, whereas the catalytic activity for smaller molecules such as toluenesulphonylarginine methyl ester (TAME) is preserved [17–19]. *In vitro* pl-Kal is inhibited by benzamidine and soy-bean-trypsin-inhibitor (SBTI) [10, 20], but after formation of the α_2 -M complex, the TAME esterase activity (TAME-ea) of pl-Kal is only inhibited by benzamidine because of the small size of the benzamidine molecule. SBTI is too large to interact with the pl-Kal molecule in the complex and thus can not inhibit TAME-ea [10, 20].

In vivo, t-Kal is only slowly and weakly inhibited by α_1 -antitrypsin [21], whereas *in vitro* it can also be inhibited by aprotinin, which is more effective [22, 23]. To investigate whether different mechanisms of kinin generation are involved in the acute and chronic forms of lung inflammation, bronchoalveolar lavage (BAL) was performed in patients with acute pneumonia and in patients with chronic bronchitis as well as in healthy controls.

Kinins, pl-Kal, α_2 -M and TAME-ea were measured in the lavages. Since TAME is a fairly nonspecific substrate, gel filtration chromatography was performed to further characterize the proteins with TAME-ea. α_2 -M and pl-Kal were also measured in these fractions. After chromatography, benzamidine, aprotinin and SBTI were added to the fractions to inhibit the TAME-ea for further characterization.

Materials and methods

Subjects

Twelve patients with acute pneumonia (six female, six male; mean age 67.8 yrs) were studied. Acute pneumonia was determined clinically and verified by chest radiography, bronchoscopy and the presence of an infecting organism. In addition, 11 patients (four female, seven male; mean age 53.5 yrs) with chronic bronchitis (according to the Medical Research Council (MRC) definition) diagnosed by history were studied. None had a history or evidence of an acute infection. All patients were non-smokers, and cardiac, renal and hepatic failure as well as systemic diseases were excluded.

Eight subjects (four female, four male; mean age 59.5 yrs) were used as normal controls. Bronchoscopy in these subjects was performed to exclude the presence of a lung tumour. The study was approved by the local Ethics Committee and patients were asked for their permission to perform scientific evaluation of BAL fluid (BALF) parallel to the routine work-up for establishing the diagnosis.

Materials

Rabbit anti-human pl-Kal and rabbit anti-human α_2 -M antisera as well as purified human pl-Kal and α_2 -M were obtained from Medor (Herrsching, Germany). Goat anti-human kinins, human bradykinin, benzamidine, SBTI, aprotinin and bovine serum albumin (BSA) were obtained from Sigma (Deisenhofen, Germany). Biotinylation kit, alkaline phosphatase conjugated streptavidin, ^3H -TAME and ^{125}I were obtained from Amersham (Braunschweig, Germany). Sephacryl S-300, DEAE-Sephadex G-100 and the gel-filtration column were obtained from Pharmacia (Freiburg, Germany).

Bronchoalveolar lavage

BAL was performed during bronchoscopic examination. One hundred millilitres of 0.9% NaCl solution were instilled in 20 mL fractions and immediately removed [24]. Ethylenediamine tetra-acetic acid (EDTA) was added in a concentration of 0.01 M. BAL samples were centrifuged at 2000×g for 10 min, the pellets were discarded and the supernatants were immediately frozen and stored at -70°C until further use.

Kinin radioimmunoassay

Kinins were measured by a specific radioimmunoassay. The assay was capable of detecting a minimum

concentration of 40 pg bradykinin·mL⁻¹. Inter- and intra-assay coefficients of variation (SEM/mean × 100%) are 2 (n=8) and 5% (n=6), respectively. The assay did not distinguish, on a molar basis, between three mammalian kinins (bradykinin, lysyl-bradykinin, and methionyl-lysyl-bradykinin). Cleavage of bonds within the kinin moiety resulted in almost complete destruction of cross-reactivity, particularly if bonds near the carboxyl terminals were affected. Highly purified human kininogen displayed less than 2% cross reaction, on a molar basis in this assay. No other cross-reacting substances are known [8].

TAME esterase activity determination

Enzymes expressing esterase activity were measured by a radiochemical assay, as described by BAUMGARTEN *et al.* [10], which is based on the liberation of ^3H -labelled methanol from the synthetic substrate ^3H -TAME.

Pre-kallikrein/Plasma kallikrein ELISA

Plasma kallikrein and pre-kallikrein (pre-Kal) were measured with a new sandwich enzyme-linked immunosorbent assay (ELISA) developed during this study. Polystyrene microtitre plates were coated, overnight at 4°C, with 100 μL ·well⁻¹ immunoglobulin G (IgG) fraction of an ion-exchange chromatography purified goat anti-human pl-Kal antiserum. Antibodies were diluted to 5 μg ·mL⁻¹ in 0.05 M carbonate buffered saline (0.05 M NaHCO₃, 0.15 M NaCl, 0.02% NaN₃, pH 9.6). Unspecific binding was blocked with 1% BSA (200 μL ·well⁻¹) in Tris-buffered saline (TBS) (0.1 M tris(hydroxymethyl)-aminomethane, 0.15 M NaCl, 0.02% NaN₃, 0.05% Tween-20, pH 7.6). One hundred microlites per well (diluted 1/20 in TBS with 0.2% BSA), or purified human pl-Kal (2–2,000 ng·mL⁻¹ in TBS with 0.2% BSA) as reference, was incubated for 1 h. One hundred microlites per well of the biotinylated rabbit anti-human pl-Kal antibody (1 μg ·mL⁻¹ in TBS with BSA) was added. This antibody was biotinylated with a "Biotinylation kit" (Amersham). After 60 min incubation, 100 μL ·well⁻¹ alkaline phosphatase-conjugated streptavidin was added (diluted 1/4,000 in TBS with BSA) and incubated for another hour. One hundred microlites per well of p-NPP (1 mg·mL⁻¹) in substrate buffer (9.7% diethanolamine, 0.1 mM MgCl₂, 0.02% NaN₃, pH 9.9) was added and optical density (OD) was determined at 405 nm on an automated micro-titre plate reader (Multiskan MCC/340 MK 11, Flow-Laboratories International SA, Lugano, Switzerland). Plasma kallikrein values were calculated by interpolation from the reference dilution curve using the P.Fit calculation program (Biosoft, Cambridge, UK). The ELISA had a lower limit of detection of 4 ng·mL⁻¹ pl-Kal⁻¹. Specificity was confirmed using purified α_2 -M, purified t-Kal, purified human IgG and human serum albumin (HSA) in concentrations between 0.5 and 50 μg ·mL⁻¹. The ELISA did not differentiate between pre-Kal and pl-Kal. The results were reproducible among replicates (coefficient of variation (CV) = 7.4±1%, n = 51), dilutions (CV = 7.3±0.7%, n = 20) and assays (CV = 9.9±0.8%, n = 5).

α_2 -macroglobulin ELISA

In principle the α_2 -M ELISA was performed in the same way as the pre-Kal/pl-Kal ELISA (same buffers, same incubation procedures) with the exception that samples were diluted 1/100 prior to the α_2 -M ELISA. The ELISA had a lower limit of detection of 20 ng·mL⁻¹ α_2 -M and no cross-reactivity was seen with purified pl-Kal, purified t-Kal, purified human IgG and purified human IgM in concentrations of 0.5–50 μ g·mL⁻¹. The results were reproducible among replicates (CV=5.3±1%, n=51), dilutions (CV=6.2±0.6%, n=20) and assays (CV=9.7±0.7%, n=5).

Gel filtration chromatography

Gel filtration chromatography was performed as described by BAUMGARTEN *et al.* [10]. Briefly, 20 mL BAL fluid was concentrated to 1.5 mL using Amicon concentrators (Centricon® concentrator 10; Amicon Inc., Beverly, MA, USA) with a 10 kDa molecular cut-off. The concentrated BAL samples were placed on a Sephacryl S-300 column (Pharmacia Biotech, Uppsala, Sweden) and eluted with Tris buffer (pH 7.4, 0.1 M TRIS, 1 M NaCl) at 10.5 mL·h⁻¹ and 1.9 mL fractions were collected. Kinins, pl-Kal, α_2 -M and TAME-ea were determined in each fraction.

TAME esterase activity inhibition

After gel filtration chromatography the TAME-ea peaks were further characterized by inhibition tests using different inhibitors as described by BAUMGARTEN *et al.* [10]. Briefly, 10⁻⁵ M benzamidine, 10⁻⁵ M SBTI or 2×10⁻⁵ aprotinin, in final concentrations, were added to the filtration fractions and incubated for 30 min. Thereafter, TAME-ea was determined as described above.

Statistical analysis

Data are expressed in the text as mean±SEM, unless otherwise specified. For comparison between different values a simple linear correlation coefficient analysis was used. The Mann-Whitney test was used for the comparison of groups. STATGRAPHICS® software (Statistical Graphics Corporation STSC Inc., Rockville, MA, USA) was used. A p-value of less than 0.05 was taken to be statistically significant.

Results

Kinin radioimmunoassay

Significantly more kinins were found in lavages from patients with acute pneumonia (mean±SEM 950±415 pg·mL⁻¹, median 743 pg·mL⁻¹) and chronic bronchitis (336±93 pg·mL⁻¹, median 311 pg·mL⁻¹) compared to the control lavages which were all below the detection level (<40 pg·mL⁻¹) (p<0.001 and p<0.01, respectively). Lavages from patients with acute pneumonia showed higher mean kinin values than lavages from patients with chronic bronchitis, but failed to reach statistical significance (64–4,840 pg·mL⁻¹ in acute pneumonia and <40–840 pg·mL⁻¹ in chronic bronchitis, p=0.11) (fig. 1a). For statistical purposes, we used the value of 20 for the kinin values <40 pg·mL⁻¹.

Fig. 1. — Values of a) kinins; b) toluenesulphonylarginine methyl-ester esterase activity (TAME-ea); c) pre-Kallikrein (pre-Kal)/plasma Kallikrein (pl-Kal); and d) alpha₂-macroglobulin (α_2 -M) in the lavages of patients with acute pneumonia (Acute; n=12), chronic bronchitis (Chronic; n=11), healthy controls (Control; n=8). Horizontal bars indicate the median for each group of patients. *, p<0.01; **, p<0.001; ***, p<0.0001; NS: nonsignificant.

TAME esterase activity

TAME esterase values were significantly higher in lavages from patients with acute pneumonia ($12,456 \pm 1,294$ counts per minute (cpm), median 14,088 cpm) and chronic bronchitis ($11,640 \pm 1,499$ cpm, median 12,105 cpm) compared to the control lavages ($1,232 \pm 245$ cpm, median 1,010 cpm) ($p < 0.001$ and $p < 0.01$, respectively). Again there was no statistical difference between patients with acute pneumonia and chronic bronchitis ($p = 0.75$) (fig. 1b).

Pre-kallikrein plasma kallikrein

Pre-kallikrein/plasma kallikrein values were significantly higher in lavages from patients with acute pneumonia (492 ± 117 ng·mL⁻¹, median 427 ng·mL⁻¹) and chronic bronchitis (78 ± 14 ng·mL⁻¹, median 66 ng·mL⁻¹) compared to the control lavages (18 ± 5 ng·mL⁻¹, median 15 ng·mL⁻¹) ($p < 0.0001$ and $p < 0.01$ respectively). In contrast to bradykinin and TAME-ea values the pre-Kal/pl-Kal values from patients with acute pneumonia were significantly higher compared to patients with chronic bronchitis ($p < 0.001$) (fig. 1c).

α_2 -macroglobulin

Patients with acute pneumonia showed significantly higher α_2 -M values (312 ± 48 μ g·mL⁻¹, median 236 μ g·mL⁻¹) than patients with chronic bronchitis (82 ± 30 μ g·mL⁻¹, median 57 μ g·mL⁻¹, $p < 0.001$) and both were significantly higher than normal controls (7 ± 1 μ g·mL⁻¹, median 7 μ g·mL⁻¹) ($p < 0.001$ and $p < 0.01$, respectively) (fig. 1d).

Gel filtration chromatography

Three main peaks of TAME-ea were observed in BAL samples after gel filtration chromatography at ~40 kDa, ~200 kDa and at ~800 kDa. In figure 2, TAME-ea, α_2 -M and pre-Kal/pl-Kal in the fractionated BAL samples from one patient with acute pneumonia and one with chronic bronchitis are shown. The results given in this figure are representative of all patients with acute pneumonia and chronic bronchitis, although there was a variation in the height of the single peaks. The highest peak of TAME-ea in BALF from patients with acute pneumonia was observed at 800 kDa which coincides with the α_2 -M peak. Furthermore, an additional α_2 -M peak in BALF from patients with acute airway inflammation was found at ~400 kDa (fig. 2b). In five patients there were four peaks positive for α_2 -M (at ~800, ~600, ~400 and ~200 kDa). Pre-kallikrein/plasma kallikrein was found as a single peak at ~100 kDa (fig. 2c). In contrast, the highest peak of TAME-ea in BALF from patients

Fig. 2. – Results from the concentrated bronchoalveolar lavages, after gel filtration chromatography of one patient with acute pneumonia (○) and one with chronic bronchitis (●). a) Toluene sulphonylarginine methyl ester esterase activity (TAME-ea). The highest TAME-ea peak in the patient with the acute pneumonia is seen at ~800 kDa. For the patient with chronic bronchitis the highest peak is seen between 24 and 60 kDa (~40 kDa). b) Alpha₂-macroglobulin (α_2 -M). The highest α_2 -M value for the patient with acute pneumonia is seen at ~800 kDa. A second peak is seen at ~400 kDa. This may be the dimer of α_2 -M. For the patient with chronic bronchitis, the α_2 -M peak is much smaller. c) Pre-kallikrein/plasma kallikrein (pre-Kal/pl-Kal) immunoreactivity is found at about 100 kDa, but for acute patients the value is much higher.

with chronic bronchitis was eluted at ~40 kDa (fig. 2a). The α_2 -M peak and the pl-Kal peak in BALF from patients with chronic bronchitis were small compared to the peaks found in acute pneumonia. In the control BALF, TAME-ea, pl-Kal or α_2 -M was not detectable after chromatography.

TAME activity inhibition

To further characterize the three main TAME-ea peaks (peak I ~800 kDa, peak II ~200 kDa and peak III ~40 kDa), four fractions with the highest TAME-ea

Table 1. – Inhibition of TAME-ea after gel filtration chromatography of BAL samples of patients with acute and chronic airway inflammation

Inhibitor	Acute pneumonia % inhibition			Chronic bronchitis % inhibition		
	Peak I	Peak II	Peak III	Peak I	Peak II	Peak III
Aprotinin 2×10^{-5} M	33±8	39±18	57±13	25±9	42±13	64±14
Benzamidine 10^{-3} M	93±5	42±17	26±11	88±13	35±14	34±12
SBTI 10^{-5} M	14±5	24±16	27±10	16±9	29±14	23±9

TAME-ea: toluenesulphonylarginine methyl ester esterase activity; BAL: bronchoalveolar lavage; SBTI: soy-bean-trypsin-inhibitor.

Fig. 3. – Correlations between the parameters measured in the lavages. Significant correlations are found between: a) kinins and TAME-ea; b) kinins and pre-Kal/pl-Kal; c) kinins and α_2 -M; and d) pre-Kal/pl-Kal and α_2 -M. ○: acute pneumonia; ●: chronic bronchitis; ▽: control. pre-Kal: pre-Kallikrein; pl-Kal: plasma Kallikrein; α_2 -M: Alpha₂-macroglobulin; TAME-ea: toluenesulphonylarginine methyl ester esterase activity.

values of each peak were mixed and the pooled fractions were incubated with different inhibitors. The first peak (~800 kDa) was strongly inhibited by benzamidine (~90%) and the third peak (~40 kDa) was inhibited up to 64% by aprotinin (table 1). No significant difference was observed between the inhibition tests in BALF from patients with acute pneumonia and from patients with chronic bronchitis.

Correlation analysis

Kinins correlated significantly with TAME-ea ($r=0.45$, $p<0.05$, $n=21$), α_2 -M ($r=0.56$, $p<0.001$, $n=21$) and pre-Kal/pl-Kal ($r=0.48$, $p<0.01$, $n=21$). A highly significant

correlation was also found between α_2 -M, and pre-Kal/pl-Kal ($r=0.92$, $p<0.00001$, $n=31$) (fig. 3). Only the BAL samples with detectable kinin values were used for the correlation analysis of kinins with pre-Kal/pl-Kal, α_2 -M and TAME-ea.

Discussion

In the present study we have found significantly higher kinin values in BALF from patients with acute pneumonia or chronic bronchitis than in healthy controls. We also demonstrated elevated TAME-ea, and thus enhanced kinin-generating activity, in acute and chronic inflammation compared to healthy controls whereas

chronic bronchitis and acute pneumonia did not differ regarding both parameters (TAME-ea and kinin). However, pl-Kal and α_2 -M values in acute pneumonia were significantly higher than in healthy controls and chronic bronchitis. After gel filtration chromatography, the highest TAME-ea values in acute pneumonia were found at a MW of 800 kDa, together with high α_2 -M values, with inhibition only by benzamidine. On the other hand, in chronic bronchitis the highest TAME-ea values were found at ~40 kDa, with strong inhibition by aprotinin, a potent inhibitor of t-Kal. Thus, in acute airway inflammation, kinins seem to be mainly generated by pl-Kal and bound to α_2 -M whereas in chronic inflammation, kininogen-ases other than pl-Kal, such as tissue kallikrein, seem to be more important.

Kinins are small peptides with a variety of biological functions such as vasodilation, bronchoconstriction and enhancement of the endothelial permeability, fibroblast stimulation and induction of pain [25–29]. In bronchoalveolar and nasal lavages, the liberation of kinins after allergen challenge or viral infections was reported [20, 30, 31], indicating their potential role in the development of airway inflammation [5]. Plasma-kallikrein and t-Kal were demonstrated to be mainly responsible for the liberation of kinins from HMW-kininogen and LMW-kininogen [5].

To evaluate whether different kinin-generating pathways are activated in different inflammatory conditions we measured TAME-ea, pl-Kal and α_2 -M in BALF. TAME-ea was used as a very sensitive marker for kinin-generating activity. Because TAME is a relatively non-specific substrate [7, 10] we further characterized the TAME-ea with gel filtration chromatography and inhibition tests. α_2 -M was measured as a potent inhibitor of pl-Kal, forming a complex with pl-Kal in which the TAME-ea of the molecule, in contrast to complexes with other inhibitors, is preserved. For the measurement of α_2 -M and pl-Kal, two sandwich ELISAs were developed. The ELISAs were sensitive enough to detect α_2 -M and pl-Kal in BALF and had good reproducibility. The results showed enhanced kinin generation in both acute and chronic airway inflammation. However, the kinin values of the BALF from patients with acute pneumonia did not differ significantly from those of patients with chronic bronchitis, but did show a wider range (64–4,840 pg·mL⁻¹ for acute pneumonia vs 20–840 pg·mL⁻¹ for bronchitis). TAME-ea values did not differ significantly between chronic and acute inflammation, but were significantly higher in both diseases than in healthy controls.

In contrast to kinin and TAME-ea values, significant differences were seen between acute and chronic inflammation concerning the pl-Kal and α_2 -M values with higher values in lavages of patients with acute pneumonia. The high TAME-ea peak, eluting at ~800 kDa, was strongly inhibited by the pl-Kal inhibitor benzamidine (up to 93%) [10, 32]. Inhibition with SBTI, however, which is another potent inhibitor of pl-Kal, was less than 20%. We interpreted the differential results of these two pl-Kal inhibitors as due to their different size. Whereas benzamidine is able to inhibit the enzymatic activity of pl-Kal even when complexed with α_2 -M due to its size (157 Da), the larger SBTI (20,000 Da) is unable to inhibit the complexed form of pl-Kal. Preliminary results, from ongoing studies of α_2 -M

and pre-Kal activation, seem to indicate that α_2 -M molecules in plasma are not normally combined with TAME-ea.

However, our lavage data suggest that activation of pre-Kal occurred in the course of the disease and that the resulting pl-Kal was bound to α_2 -M. High values for kallikrein and α_2 -M were detected in the BALF of patients with acute pneumonia, and high peaks of TAME-ea were seen at ~800 kDa after gel filtration chromatography.

Furthermore, earlier inhibition studies by BAUMGARTEN *et al.* [10] indicated that the major TAME esterase peaks in the void volume were strongly affected by benzamidine. Therefore, we believe that most of the kinin-generating activity in the present study in pneumonia was due to activation of pre-Kal to pl-Kal, which was later inactivated by inhibitors, such as α_2 -M. Similar results have been observed in allergic rhinitis and in BALF from patients after allergen challenge [8–11, 30].

In contrast, the high TAME-ea values found at ~40 kDa in chronic bronchitis, were inhibited by aprotinin (about 60%), which is a potent inhibitor for t-Kal [11, 23], and were unlikely to be due to pl-Kal activity but rather to t-Kal. BAUMGARTEN *et al.* [11] demonstrated that purified t-Kal is inhibited up to 100% by aprotinin. Thus, the part of the TAME-ea found in the third peak seems to depend on molecules similar but different from t-Kal. The partial inhibition of this peak by SBTI (27%), which does not inhibit t-Kal, would seem to confirm this suggestion.

A further indicator of the minor role of inflammation and plasma leakage in chronic bronchitis are the relatively small peaks of pl-Kal and α_2 -M seen after gel filtration chromatography. These results suggest that in chronic bronchitis, kinin generation may be mainly due to t-Kal. Studying the BALF of asthma patients, CHRISTIANSEN *et al.* [33] found the highest kininogenase activity in small molecules after gel filtration chromatography (20–34 kDa). This kininogenase profile appears similar to the profile we found in patients with chronic bronchitis. Theoretically, pl-Kal and t-Kal activity could be further differentiated with the direct measurement of bradykinin (as product of the pl-Kal activity) and lysyl-bradykinin (as product of the t-Kal activity). However, we believe that the catalytic activity of aminopeptidase N, degrading lysyl-bradykinin to bradykinin, would influence the lysyl-bradykinin measurement [34, 35].

Interestingly, kinin formation was only observed in BALF with >500 ng·mL⁻¹ pl-Kal⁻¹ and/or >80 µg·mL⁻¹ α_2 -M⁻¹. This is likely to reflect the necessary substrate presentation. The pre-Kal/pl-Kal ELISA shows only one peak (at ~100 kDa) in the fractions of the chromatography. This peak was interpreted as pre-Kal because the antibodies did cross react. Plasma kallikrein itself is not very likely to be detected by this ELISA because it is complexed with inhibitors such as α_2 -M and thus masks binding sites necessary for the detection antibodies. As shown in figure 2, α_2 -M immunoreactivity was found in two peaks at ~800 and 400 kDa in acute pneumonia. In some cases we found additional peaks at ~600 and 200 kDa (data not shown). This may be due to the occurrence of monomers, dimers, trimers and tetramers with MWs of 180, 360, 540 and 725 kDa. The native function of these α_2 -M molecules needs to be further investigated.

In conclusion, the kinin generation found in acute inflammation seen in pneumonia seems to be mostly dependent on plasma kallikrein, whereas in chronic bronchitis the kinin generation seems to follow an alternative pathway with formation of kinins by tissue kallikrein. These data confirm the concept that pneumonia is a disease with a major vascular inflammatory component and protein and fluid leakage, whereas in chronic bronchitis the involvement of the local mucosal tissue structures, like glands, seem to be more important for inflammation. Unfortunately, very little has been published concerning different kallikrein activation pathways and their effect on inflammation and plasma leakage in acute and chronic diseases of the lung. The current study indicates that different pathways may be involved.

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